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- a. incorporating a nucleotide analog including 2-aminopurine into a probe;
- b. affixing the labeled or modified probe on a substrate;
- c. detecting a first level of label expressed by the labeled or modified probe molecules on the substrate;
- d. contacting substrate with a volume of sample containing unlabeled or unmodified target molecules in solution;
- e. providing sufficient conditions and time for unlabeled or unmodified target molecules in solution to selectively pair and hybridize with the labeled probe molecules affixed on the substrate;
- f. removing the substrate and detecting the second level of label expressed by the labeled probed affixed on the substrate after exposure to the unlabeled or unmodified target molecules in solution;
- g. comparing the first and second levels of label expressed by the labeled or modified probe;
- h. identifying probe and target hybridized molecules by repeating steps c-f until the amounts of label expression between the first and second levels of label approaches zero and/or about background levels.

### REMARKS

#### **I. PRELIMINARY REMARKS**

Applicant has carefully considered the detailed Office Action and sets forth detailed responses herein. Applicant has amended specific claims addressed by the Examiner in the Office Action dated October 3, 2001.

Claims 3, 8 and 21 have been cancelled.

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Claim 9 was previously cancelled in the last amendment.

Claims 17, 22-25 and 27-30 have been amended to more particularly point out and distinctly claim certain aspects of Applicant's invention. The amended claims ***do not introduce new subject matter*** because the essence and language of each claim can be found in the disclosure as filed.

Claims 33-37 have been added. Claims 33-37 do not raise new issues or introduce new subject matter; rather they more particularly point out and distinctly claim certain unique aspects of the Applicant's invention.

Reconsideration and allowance of all of the claims in view of the above amendments and the following remarks are respectfully requested.

## **II. SYNOPSIS OF THE PRESENT INVENTION**

The present invention provides an assay system using labeled probe molecules by incorporation of nucleotide analogs to identify and quantify unlabeled and unmodified target molecules in solution or sample. In one embodiment, labeled probe molecules are present on a solid support or substrate such as a microarray, and upon contacting the labeled probe with an unlabeled/unmodified target in solution, the identification of multiple different unlabeled/unmodified target molecules is made simultaneously. In another preferred embodiment, incorporation of fluorescent single stranded nucleotide analogs, including 2-Amino purine, into probes causes reduced fluorescence or quenching when paired or hybridized with a homologous unlabeled or unmodified target molecule in solution.

## **III. THE REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH**

Claims 17, 22-25 and 27-30 are rejected under the second paragraph of 35 U.S.C. § 112 as being indefinite for failing to particularly point out and distinctly claim the subject matter, which the applicant regards as the invention.

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Applicant respectfully traverses this rejection. The claims have been amended to make them more clear and definite, this amendment is being made in order to recite the methods and steps explicitly and obviate any possible basis for rejection on the grounds of indefiniteness.

Further, it is not the function of the claims to describe every possible dimension or quantity of a claimed feature. Orthokinetics, Inc. v. Safety Travel Chairs, Inc., 1 U.S.P.Q. 2d 1081 (Fed. Cir. 1986). The question is whether the claims are as precise as the subject matter permits and reasonably apprise those skilled in the art as to the metes and bounds of the claim. Hybritech, Inc. v. Monoclonal Antibodies, Inc., 231 U.S.P.Q. 81 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987).

Applicant believes that the metes and bounds of the claims are clearly set forth.

Specifically, claim 17 has been amended in paragraph (c) to recite "contacting the first substrate."

Claims 22-25 are amended to recite a positive method step.

Claims 27-30 are amended to remove the reference to "the second level." Claim 30 is also amended to remove the references to "complementary target level" and "the second level approaches zero and the second level is greater than zero."

Accordingly, the Examiner is respectfully requested to withdraw these rejections.

IV. THE REJECTIONS UNDER 35 U.S.C. § 102(E) AS BEING ANTICIPATED BY U.S. PATENT NO. 6,100,030 TO McCASKY ET AL. (McCASKY ET AL. '030)

1. Rejection of Claims 1-2 and 22-31.

It is the Examiner's position that McCasky '030 teaches an array containing a labeled probe as described by the present invention.

McCasky '030 does not anticipate claims 1-2 and 22-31. In short, McCasky '030 does not teach the claimed methods. What McCasky '030 does teach are methods of genotyping

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amplified mixtures of DNAs that do not rely on gel-electrophoresis for the generation of DNA footprints (col. 3, lines 24-25). To generate a DNA fingerprint, a polynucleotide probe, made according to the method of the invention, is hybridized to a mixture of amplified DNA generating a positive or a negative hybridization result (col. 3, lines 37-41).

- a. **McCasky '030 does not teach or suggest labeling probes by incorporation of nucleotide analogs and affixing the labeled probe on a substrate.**

The Examiner rejects claims 1-2 and 22-31 based primarily on McCasky '030, column 23, and lines 40-51. According to the Examiner Claim 1 (the substrate comprises attached labeled molecules) is rejected because McCasky '030 in column 23, line 40 states that a "probe is linked to a solid support and a target nucleic acid (e.g., a genomic nucleic acid, an amplicon, or, most commonly, an amplified mixture) is hybridized to the probe." Claim 2 (labeled probe is fluorescent) is rejected because McCasky '030 states that "[e]ither the probe, or the target, or both, can be labeled, typically with a fluorophore." Claims 22-25 are rejected because McCasky '030 states that "[w]here the probe is labeled, hybridization is typically detected by quenching of the label."

However, in column 23, lines 57-61, McCasky '030 recites that "[using] chip masking technologies and photoprotective chemistry it is possible to generate ordered arrays of nucleic acid probes. These arrays, which are known e.g. as "DNA chips," or as very large scale immobilized polymer arrays ("VSLIPS<sup>TM</sup>" arrays) can include millions of defined probe regions on a substrate..." (Fodor et al., (1991) Science 251:767-777).

Further, in column 24, lines 36-41, McCasky '030 states that "... one of skill in the art is able to order custom-made arrays and array-reading devices from manufacturers specializing in array manufacture. For example, Affymetrix Corp., in Santa Clara, Calif. manufactures DNA VLSIP<sup>TM</sup> arrays.

Moreover, Fodor is the inventor in many of the Affymetrix patents, which relate to many aspects of microarray technology. *However*, Affymetrix methods are based on *labeling a target*,

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not labeling of an affixed probe on a substrate or array, which differentiates Affymetrix methods from that of the present invention.

The present invention *does not label targets*, in the present invention the targets are unlabeled or unmodified. The present invention *labels probes* by incorporating a nucleotide analog fluorophore and then affixing the labeled probes onto a substrate or array. Thus, based on McCasky's analogy of Affymetrix' DNA chip or VLSIPS™, there is no description of labeling a probe affixed on a substrate, because the Affymetrix DNA chips and VLSIPS™ affix unlabeled probe to substrate. In Affymetrix "[t]he *target polynucleotide may be labeled* by any of a number of convenient detectable markers...The detecting of the positions which bind the target sequence would typically be through a *fluorescent label on the target* (United States Pat. No. 6,197,506 to Fodor et al., Assignee, Affymetrix, Inc. (Santa Clara, CA)).

Thus, McCasky '030 *does not teach or suggest* labeling probes by incorporation of a fluorescent nucleotide analog and affixed to a substrate. Further, McCasky '030 describes analogous methods disclosed by Affymetrix, which also lacks any teaching or suggestion of labeling probes affixed to a substrate. Hence, in the absence of any teaching or suggestion in either the McCasky '030 reference or by Fodor et al., anticipation of the present invention is negated. This is because all elements of the claimed invention must be disclosed in a single reference for anticipation to exist. Atlas Powder Co. v. E. I. DuPont de Nemours & Co., 750 F.2d 1569, 224 U.S.P.Q. 409 (Fed. Cir. 1984). Missing elements cannot be supplied by the knowledge of one skilled in the art or the disclosure of another reference in order to give rise to an anticipation rejection. Structural Rubber Products Co. v. Park Rubber Co., 749 F.2d 707, 223 U.S.P.Q. 1264 (Fed. Cir. 1984). Accordingly, the absence of any teaching or suggestion in McCasky '030 of labeling probes affixed on a substrate means that there is no anticipation rejection.

McCasky '030 by chance states one aspect of the claimed invention, that "[e]ither the probe, or the target, or both, can be labeled, typically with a fluorophore." However, the reference does not disclose the incorporation of a nucleotide analog fluorophore into a probe. In fact, the scope and content of the McCasky '030 patent is completely differently than the present invention. Additionally, the *art must have existed as of the date of the invention*, presumed to

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be the filing date of the application (June 9, 1998) until an earlier date is proved. The Applicant's own search, at the time of filing of the McCasky '030 patent, and even to the present time, shows that there is no literature describing the methods and various novel aspects of the present invention. That is, the present invention is the earliest date whereby incorporation of a fluorophore into a probe affixed on a substrate is described, not McCasky '030. Thus, while McCasky '030 may suggest that "[e]ither the probe, or the target, or both, can be labeled, typically with a fluorophore," McCasky '030 does *not describe or suggest* that the incorporation of a fluorophore such as 2-Aminopurine into a probe affixed on a substrate, and therefore does not motivate or anticipate the claimed invention. In re Baird, 29 U.S.P.Q. 2d 1550 (Fed. Cir. 1994).

In short, the scope and content of the present invention is a method in which incorporation of a fluorophore, including 2-aminopurine, into a probe and then affixed on a substrate (Claims 20 and 33-35). The labeled or modified probe is then hybridized to its complementary unlabeled or unmodified target molecules in solution (Claims 20, 22-25 and 34-35). This method is simply not taught in McCasky '030, which teaches methods of genotyping amplified mixtures of DNAs that do not rely on gel-electrophoresis for the generation of DNA footprints (col. 3, lines 24-25).

**b. Labeling probes by incorporation of nucleotide analogs and affixing on a substrate is not inherent in McCasky '030.**

Also, "[a]nticipation is established only when a single prior art reference discloses expressly or under the principles of inherency, each and every element of the claimed invention." RCA Corp. v. Applied Digital Data Systems, Inc., (1984, CAFC) 221 U.S.P.Q. 385. The standard for lack of novelty, that is, for "anticipation," is one of strict identity. To anticipate a claim, a patent or a single prior reference *must contain all the essential elements of the particular claims*. Schroeder v. Owens-Corning Fiberglass Corp., 514 F. 2d 901, 185 U.S.P.Q. 723 (9<sup>th</sup> Cir. 1975). In the present Office Action, the Examiner's rejection is primarily based on McCasky '030 reference, which *fails to show all the essential elements* of the instant invention.

Labeling of probes by incorporation of nucleotide analogs and affixing the labeled probes on a substrate *is not inherent* in McCasky '030. The only time McCasky '030 mentions that

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probes can in fact be labeled is in column 23, line 44. Yet, McCasky '030 **does** not disclose any method of labeling a probe and affixing to a substrate. McCasky '030 discloses that arrays of probes can be purchased from Affymetrix. However, Affymetrix does not manufacture or supply *labeled probe* arrays; they supply *unlabeled probe* arrays. Hence, the standard for inherency in the context of anticipation has not been met.

"Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient." Continental Can Co. USA, Inc. v. Monsanto Co., 948 F.2d 1264, 1268-69, 20 U.S.P.Q. 2d 1746, 1749 (Fed. Cir. 1991) (quoting In re Oelrich, 666 F.2d 578, 581, 212 U.S.P.Q. 323, 326 (C.C.P.A. 1981)). In short, *there must be factual and technical grounds establishing that the inherent feature necessarily flows from the teachings of the prior art.* Ex parte Levy, 17 U.S.P.Q. 2d 1461, 1464 (Bd. Pat. App. & Int.'f 1990).

By the standard above, inherency is not present in McCasky '030. The required "factual and technical grounds" for inherency are lacking in McCasky '030. The Examiner has made no showing that McCasky '030 discloses methods of labeling probes affixed on a substrate. There is **no suggestion** that "either the probe, or the target...can be labeled..." as taught in McCasky '030 is analogous to the methodologies of the present invention. The methodologies of the McCasky '030 and the present invention are **not analogous**.

Accordingly there is no anticipation of these claims.

2. **Rejection of Claims 8 and 9**

Claims 8 and 9 have been cancelled.

V. **THE REJECTIONS UNDER 35 U.S.C. § 103(A) AS BEING UNPATENTABLE OVER McCASKY '030**

1. **Rejection of Claim 3**

The Applicant respectfully asserts that this rejection is now moot in light of the cancellation of Claim 3.

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2. **Rejection of Claims 4-5, 10-13, 16-17, 19-20 and 32 as being unpatentable over McCasky '030 in view of McGall '501.**

In the present invention Claim 4 is drawn to a labeled probe comprised of native and nonnative nucleotides. Claim 5 is drawn to nucleotides being nucleotide analogs. Claims 10-12 are drawn to microarrays being divided into quadrants wherein each different quadrant has labeled probe molecules of different sequences. Claim 13 is drawn to a microarray being a bead. Claims 16-17 and 19-20 are drawn to methods comprising detecting a difference in probes before and after hybridization. Claim 32 is drawn to the method of Claim 16, wherein multiple labeled probes and the multiplying of the labeled probes are achieved by non-amplification steps.

The Examiner concedes that McCasky '030 *does not teach* nucleotide analogs (Claim 5), or arrays divided into quadrants (Claims 10-12), or methods wherein the levels of label are expressed twice and compared (Claims 16-17, 19-20, and 22-25), or labeled probes achieved by non-amplification steps (Claim 32).

a. **McCasky '030 does not teach or suggest any aspect of the present invention.**

Applicant respectfully traverses these rejections. The guidance given in McCasky '030 is insufficient because the methods disclosed in the present invention were not even postulated in McCasky '030. Thus, based on the disclosure of McCasky '030, one of ordinary skill in the art would have no basis for labeling the probes instead of labeling the targets as analogized by McCasky '030 without the guidance presented by the disclosure of the present invention.

The use of the guidance of the claimed invention in an obviousness determination is hindsight. It has been specifically proscribed by the Federal Circuit in the determination of the existence or nonexistence of a prima facie case of obviousness. Interconnect Planning Co. v. Feil, 774 F.2d 1132, 227 U.S.P.Q. 543 (Fed. Cir. 1985).

Again, there is no discussion or guidance in the specification of McCasky '030 about labeling of probes by incorporation of nucleotide analogs, besides the one very short statement in column 23, line 43-44 stating that "[e]ither probe, or the target, or both can be labeled." There is



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simply *no guidance* (i.e. no working example) provided for labeling of probes affixed to a substrate as required by In re O'Farrell, 853 F.2d 894, 7 U.S.P.Q. 2d 1673 (Fed. Cir. 1988).

**b. There is no prima facie case of obviousness as a matter of fact.**

Further, Applicant maintains that no prima facie case of obviousness for these claims is created by this combination of references of McCasky '030 and McGall '501. This is because in the present invention, the method relies on the fact that probes have to be labeled or modified prior to affixing them onto a substrate (Claims 1, 4-6, 20, 31-33). This significant requirement is not taught or suggested by either reference alone or in combination.

The prior art whether taken individually or in combination fails to suggest labeling of probes affixed to a substrate. First, the teachings of McCasky '030 have been discussed above. Secondly, McGall '501 teaches *labeling of the target molecules in solution* with a label such as fluorescein (column 12, lines 38-47). McGall '501 provides no guidance for using *labeled probes* immobilized on a solid support that are then subsequently hybridized with their homologous *unlabeled targets in solution*. Thus, one of ordinary skill in the art would, therefore, have no incentive to carry out the methods of the present invention by relying upon the teachings of McCasky '030 in view McGall '501 because there are *no teachings of any particular methods of labeling probes* affixed on a substrate. Therefore, one of ordinary skill in the art would have no basis for concluding, *until the results presented by Applicant in the specification were known*, that that labeling a probe affixed to a substrate would be functional and would be an effective assay.

There must be a reasonable probability of success, taught by the prior art, of labeling probes affixed to a substrate in McCasky '030 alone or in combination with McGall '501. In re O'Farrell, 853 F.2d at 894, 7 U.S.P.Q. 2d at 1673. That reasonable probability of success is completely lacking in McCasky '030, because McCasky '030 only refers to labeling of targets analogous to that disclosed in detail in Fodor et al patents (Assignee, Affymetrix Corp.,). McCasky '030 teaches that DNA chips or VLSIP™ as described by Fodor et al., (1991) and manufactured by Affymetrix can be used. However, as stated above Affymetrix methods *label targets*, Affymetrix *does not label probes*. Labeling of probes is claimed in the present invention. Additionally, McGall '501 teaches that "hybridization is detected by *labeling a target*

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with e.g., fluorescein or other known visualization agents and incubating the target with an array of oligonucleotide probes (column 12, lines 39-42).” This is in *complete contrast* to the present invention, which claims methods for *labeling probes NOT targets*. Hence, in the absence of guidance for reasonable possibility of success of *labeling of probes* affixed to a substrate in either reference alone or in combination, there is no prima facie case of obviousness.

The scope and content of the prior art and the differences between the prior art and the claims at issue have been previously discussed.

**c. There is no prima facie case of obviousness as a matter of law.**

Further, obviousness under 35 U.S.C. § 103 is a question of law based on the underlying factual inquiries set forth in Graham v. John Deere Co., 383 U.S. 1, 17, 148 USPQ 459, 467 (1966): (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the art; and (4) objective evidence of secondary considerations.

“The issue of obviousness is determined entirely with reference to a hypothetical ‘person having ordinary skill in the art.’ It is only that hypothetical person who is presumed to be aware of all the pertinent art. The actual inventor’s skill is irrelevant to this inquiry, . . . The statutory emphasis is on a person of ordinary skill. Inventors, as a class, according to the concepts underlying the Constitution and the statutes that have created the patent system, possess something, which sets them apart from the workers of ordinary skill, and one should not go about determining obviousness under § 103 by inquiring into what patentees (i.e., inventors) would have known or would likely have done, faced with the revelation of references.” [Emphasis in original.] Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc., 230 U.S.P.Q. 416 (Fed. Cir. 1986).

Hence, based on the hypothetical person of “ordinary skill in the art” at the time the invention was made, it is not foreseeable that one of ordinary skill in the art could have performed the claimed invention based on the scope and content of McCasky ‘030 and reading column 23, lines 43-44; stating that “[e]ither the probe, or the target, or both, can be labeled, typically with a fluorophore.” Also, the art must have existed at of the date of the invention. Applicants position is that no such art existed at the time of McCasky ‘030 invention, and any art

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disclosed and described is in the Applicant's invention and not that of any prior art including McCasky '030.

Thus, it is the Applicant's position that the Examiner has engaged in improper hindsight analysis to conclude that the claimed invention would have been obvious. As the former Court of Customs and Patent Appeals held: It is impermissible within the framework of § 103 to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one skilled in the art. In re Wesslau, 353 F.2d 238, 241, 147 USPQ 391, 393 (CCPA 1965); see also In re Mercer, 515 F.2d 1161, 1165-66, 185 USPQ 774, 778 (CCPA 1975).

Therefore, the Examiner has improperly determined that the claimed invention is obvious because not all the Graham inquiries have been satisfied; the Examiner has not satisfied the burden of proof focusing on what is obvious to the one of ordinary skill in the art at the time the invention was made; and the Examiner has engaged in hindsight analysis to pick and choose from any one reference only so much of it as will support a given position is also not proper.

**3. Rejection of Claim 6 as being unpatentable over McCasky '030 in view of McGall '501 and further in view of Gelfand '375.**

Dependent claim 6 is indirectly dependent on independent claim 1, which provides for labeled probe molecules. Dependent claim 6 further states that the labeling molecule is 2-amino purine.

First, McCasky '030 and McGall '501 teach methods of *labeling targets* and NOT probes. Secondly, whereas Gelfand '375 labels probes (column 12, lines 35-40) using 2-amino purine, it is the standard molecular biology use of the word "probe", similar to that used in Northern and Southern blots, and not the same meaning of the word probe as used in the present invention and in McCasky '030 or McGall '501. In Gelfand '375, probes are not affixed on a substrate—probes are in solution. Probes incorporating 2-amino purine in Gelfand '375 are free floating in solution and bind to homologous *unlabeled nucleotides* linked to nitrocellulose or similar substrates. Hence, probes by this definition are equivalent to the "targets" in the present invention as well as to the "targets" in that of McCasky '030 and McGall '501.

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Thus, similar to the above arguments regarding rejections of Claims 4-5, 10-13, 16-17, 19-20 and 32, there is no prima facie case of obviousness based on McCasky '030 alone or in combination with either McGall '501 or in combination of McGall '501 in view of Gelfand '375.

**4. Rejection of Claim 7 as being unpatentable over McCasky '030 in view of McGall '501 and further in view of Scholin '530.**

Claim 7 is dependent on Claim 1, wherein the labeled probes are comprised of amino acids affixed to a substrate.

First, McCasky '030 and McGall '501 teach methods of *labeling targets* and NOT probes. Secondly, whereas Scholin '530 teaches the use of amino acids (i.e. antibodies) as probes, the use of antibodies as probes in Scholin '530 is not analogous to the present invention and its use of labeled probes. Specifically, Scholin '530 does not teach the use of labeled amino acids or polypeptides attached to a solid support in such a manner that when a target molecule binds specifically to the labeled amino acid or polypeptide, a signal is modulated or modified.

Further, as stated above, in Scholin '530, and again in Gelfand '375, the word "probe" is the standard molecular biology use of the word "probe"; similar to that used in Northern and Southern blots, and not the same meaning of the word probe as used in the present invention and in McCasky '030 or McGall '501.

This terminology of "probe" and "target" is confusing. However, before microarray technology the use of the term "probe" was standard and universally understood in the field of molecular biology. In fact, when dealing with standard molecular biology techniques, no one skilled in the art ever questions what is the definition of a labeled probe. For example, a probe is a general term for a piece of DNA or RNA corresponding to a gene or sequence of interest, that has been labeled either radioactively or with some other detectable molecule, such as biotin, digoxigenin or fluorescein. A probe will label viral plaques, bacterial colonies or bands on a gel that contain the gene of interest. On the other hand, standard molecular biology did not typically use the term "target". However, a "target" in the standard molecular biology would mean the complementary DNA or RNA which are generally cross-linked on a substrate (i.e. nitrocellulose) that hybridizes or binds to the labeled probe.

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For example, Southern blots are techniques used for searching for a specific DNA fragment. The process occurs by separating DNA fragments by gel electrophoresis; changing the pH of the gel to basic and allowing disruption of H-bonds so DNA fragments become single stranded; blotting the gel with nitrocellulose substrate; cross-linking the DNA fragments to the nitrocellulose substrate; and then **probing** with the labeled RNA or cDNA, which will hybridize with their complementary RNA or DNA. So, typically in molecular biology techniques (i.e. Northern and Southern blots), the probes are labeled and in solution.

In Scholin '530 antibody probes are not affixed to a substrate; rather the labeled antibody probes in Scholin '530 are free floating in solution and bind to homologous **unlabeled proteins** linked to nitrocellulose or other substrates. Scholin '530 is the standard molecular biology use of the word probe. In fact, Scholin '530 does not refer to the unlabeled proteins linked to nitrocellulose as "targets." That is because the use of the terms "targets" and "probes" did not exist until the advent of microarray technology. With the microarray technologies, protocols clearly define "targets" and "probes," so that there is no confusion. For example, in the present invention, "targets" are in a solution or sample. That is, "target" solutions are a not a homogenous mixture of nucleic acids, proteins, etc., Target solutions may simply contain a homologous complementary stretch of nucleic acids, proteins, etc., to the labeled probe. This definition may sound simple, however, it was never applied until the use of microarray technology. In the present invention, as well as in McCasky '030 and McGall '501, the use of the terms "target" and "probe" are the same and well defined. However, as mentioned previously, McCasky '030 *does not teach labeled probes affixed on a substrate* and McGall '501 *teaches labeling of targets and not probes*.

Thus, similar to the above arguments regarding rejections of Claims 4-5, 10-13, 16-17, 19-20 and 32, there is no prima facie case of obviousness based on McCasky '030 alone or in combination with either McGall '501 or in combination of McGall '501 in view of Scholin '530.

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5. Rejection of Claims 14 and 15 as being unpatentable over McCasky '030 in view of McGall '501 and further in view of Mandecki '571.

Claims 14 and 15 are dependent claims drawn to a substrate of Claim 1 being comprised of a ferromagnetic core and having from about 100 to about 1000 labeled probe molecules attached to the surface area of the bead.

First, McCasky '030 and McGall '501 teach methods of *labeling targets* and NOT probes. Secondly, whereas Mandecki '571 teaches beads comprising of ferromagnetic metal materials, Mandecki '571 does not teach that labeled probes are affixed to the beads comprised of ferromagnetic cores. In column 1, lines 20-26, Mandecki '571 teaches that the beads made up of ferromagnetic material are used to trap analytes and facilitate separation from complex suspension mixtures. This is not analogous to the present invention, whereby labeled probes affixed to a bead comprising of a ferromagnetic core is used to specifically bind or hybridize to homologous nucleotide sequences on targets. In the present invention, there is not a complex suspension of mixtures. In the present invention, there is simply a sample of unlabeled targets added to labeled probes affixed on a substrate such as a bead. Thus, since McGall '501 and McGall '530 and Mandecki '571 do not teach any element of dependent claims 14 and 15, alone or in combination, there is no 35 U.S.C. §103(a) rejection of obviousness.

6. Rejection of Claim 18 as being unpatentable over McCasky '030 in view of McGall '501 and further in view of Heagy '516.

Claim 18 is drawn to methods of claim 10 wherein label can be quantified using a flow cytometer.

First, McCasky '030 and McGall '501 teach methods of *labeling targets* and NOT probes. Secondly, Heagy '516 teaches the use of flow cytometry in assays allowing "one to identify opiate agonists/antagonists that bind to the lymphocyte receptors and those that bind to neurons or to both cell types (column 10, lines 23-26)." Again, this is not analogous to the present invention because in Heagy '516, labeled probes using a fluorophore such as 2-amino purine are not affixed to any substrate, or cell type. Typically, flow cytometers are used with assays that require cells in suspension, as is the case in Heagy '516. In contrast, the usage of a

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flow cytometer in the present invention is novel because the labeling of probes affixed to a substrate is not on a cell type. In the present invention, a flow cytometer because it detects fluorescence in suspensions, and applied to the present invention, a fluorescently labeled probe affixed to a substrate such as beads can be categorized as a suspension mixture.

Thus, similar to the above arguments regarding rejections of Claims 4-5, 10-13, 16-17, 19-20 and 32, there is no prima facie case of obviousness based on McCasky '030 alone or in combination with either McGall '501 or in combination of McGall '501 in view of Heagy '516.

#### VI. IN SUMMARY

Claims 3, 8 and 21 have been cancelled.

Claim 9 was previously cancelled in the last amendment.

Further, Claims 5, 17, 22-25 and 27-30 have been amended to improve their form, but do not add new subject matter.

Claims 33-37 have been added to particularly point out and distinctly claim novel aspects of the invention including: 1) incorporation of nucleotide analogs such as 2-aminopurine into probes; 2) affixing the labeled and modified probes on a solid substrate such as an array; 3) detecting a first level of fluorescence of the labeled/modified probe; 4) hybridizing the labeled/modified probe to its homologous but unlabeled and unmodified targets in solution; 5) detecting a second level of fluorescence of the labeled/modified probe hybridized to its unlabeled/unmodified target homologue; and 6) identifying a probe/target pair by differences in the levels of labeled probe the first time as compared to the levels of labeled probe the second time. All aspects of this method are not "typical" and are not taught in McCasky '030 or any other cited reference.

Thus, in view of the discussion above, there is no basis for a rejection under 35 U.S.C. § 102 or § 103 in the alternative. An alternative rejection under § 102 for anticipation or for § 103 for obviousness is proper only when the prior art discloses a product, or, by analogy, a process that "reasonably appears to be identical with or only slightly different than a product [or process] claim." In re Brown, 459 F.2d 531, 535, 173 U.S.P.Q. 685, 688 (C.C.P.A. 1972) (as applied to product claims in product-by-process form).

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The requirement for such a rejection in the alternative is not met because no labeling of probes affixed on a substrate is either disclosed or suggested in McCasky '030. Accordingly, there is no basis for such a rejection in the alternative.

Moreover, one cannot use the instructions of the present invention to piece together the teachings of the prior art. More particularly, one cannot use hindsight reconstruction to pick and choose among isolated disclosures to depreciate the claimed invention. In re Fine, 837 F.2d 1071, 1075, 5 U.S.P.Q.2d (BNA) 1596, 1600 (Fed. Cir. 1988).

## VII. CONCLUSION

In conclusion and in view of the above, it is submitted that this application is now in good order for allowance, and such early action is respectfully solicited. Should matters remain which the Examiner believes could be resolved in a telephone interview, the Examiner is requested to telephone the Applicant's undersigned attorney.

Please charge any additional fees or credit overpayment to Deposit Account No. 16-2230.

Respectfully submitted,



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## ADDENDUM PAGES

**SET OF MARKED-UP CLAIMS WITH UNDERLINING AND BRACKETS**

1. A substrate having a surface area, the surface area comprising attached labeled probe molecules
2. The substrate of claim 1 wherein the labeled probe is fluorescent.
3. (Cancel) The substrate of claim 1 wherein the labeled probe fluoresces at a wavelength of about 300 nm to about 700 nm.
4. The substrate of claim 1 wherein the labeled probe is comprised of native and nonnative nucleotides.
5. (Amended) The labeled probe molecules of claim 1 wherein the nucleotides are nucleotide analogs including 2 -amino purine [at least] for adenosine or guanine; ribonucleoside or 2,6-diamino ribonucleoside, formycin A, formycin B, oxyformycin B, toyocamycin, sangivamycin, pseudouridine, showdomycin, minimycin, pyrazomycin, 5-amino-formycin A, 5-amino-formycin B or 5-oxo-formycin A at least for adenosine; 4-amino-pyrazolo [3,4d] pyrimidine, 4,6-diamino-pyrazolo [3,4d] pyrimidine, 4-amino-6-oxo-pyrazolo [3,4d] pyrimidine, 4-oxo-pyrazolo [3,4d] pyrimidine, 4-oxo-6-amino-pyrazolo [3,4d] pyrimidine, 4,6-dioxo-pyrazolo [3,4d] pyrimidine, pyrazolo [3,4d] pyrimidine, 6-amino-pyrazolo [3,4d] pyrimidine or 6-oxo-pyrazolo [3, 4d] pyrimidine [at least] for cytosine or thymidine
6. The labeled probe molecules of claim 2, wherein the nucleotide analog is 2-amino purine.
7. The substrate of claim 1 wherein the labeled probe molecules are comprised of amino acids.
8. (Cancel) The substrate of claim 1 wherein the labeled probe molecules are comprised of carbohydrates.
9. (Cancel) The substrate of claim 1 wherein the substrate is a microarray.

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10. The substrate of claim 1 wherein the substrate is a microarray further having the surface area divided into quadrants wherein each different quadrant has different labeled probe molecules.

11. The microarray substrate of claim 10 having from about 100 to about 10,000 different labeled probe [molecule] molecules located upon about 100 to about 10,000 different quadrants.

12. The microarray of claim 10 having about 100 to about 1,000 labeled probe molecules per quadrant.

13. The substrate of claim 1 wherein the substrate is a bead, said bead sizes range from about 10 microns to about 20 microns.

14. The bead substrate of claim 13 wherein the bead is formed of a ferromagnetic metal core and a polymeric coating.

15. The bead substrate of claim 13 having from about 100 to about 1,000 labeled probe molecules attached to the surface area of the bead.

16. A method for assessing the presence of a target molecule in a cell or tissue sample comprising the steps of:

- a. procuring a microarray having a surface area comprising attached labeled probe molecules in quadrants;
- b. detecting the level of label expressed within each quadrant a first time;
- c. applying a sample comprising unlabeled target sequences to the microarray;
- d. providing sufficient conditions and time for target molecules to selectively pair with the complementary labeled probe molecules; and
- e. detecting the level of label expressed within each quadrant a second time;

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f. comparing the levels of label expressed between the first time and the second time for each quadrant.

g. repeating steps c - f until the levels of label approaches zero and/or about background levels;

h. the difference between levels of label in that of step f and that of step c identifies a target/probe pair.

17. (Amended) A method for quantifying the amount of a target molecule in solution [a sample] comprising the steps of:

a. procuring a first substrate having a surface area comprising a known number of labeled probe molecules;

b. detecting [the] a first level of label expressed by the labeled probe molecules on the first substrate;

c. contacting [a] the first substrate with a volume of sample containing unlabeled target nucleotide sequences;

d. providing sufficient conditions and time for unlabeled target molecules to selectively pair with the labeled probe molecules;

e. removing the first substrate [from the sample] and detecting the level of label expressed by the substrate after exposure to the sample containing unlabeled target molecules;

f. where the level of label expression of the first substrate is substantially reduced to levels substantially similar to background levels, repeating steps a. through e. with subsequent substrates, having surface areas comprising [a] known numbers of labeled probe molecules.

g. calculating the amount of target molecule in the volume of sample by adding the known number of labeled probe molecules present on the first substrate and subsequent substrates contacted with the sample, wherein the levels of label expression of the substrates were reduced relative to the levels prior to contacting the sample.

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18. The method of claim 10, wherein the level of label expression is evaluated using a flow cytometer.

19. A substrate having a surface area divided into quadrants;

different nucleotide probe molecule sequences bound to the surface area, wherein different nucleotide probe molecule sequences are bound to distinct quadrants;

wherein the nucleotide probe molecules are characterized as being a single stranded form or double stranded in form, wherein the level of label expressed from the single stranded probe molecules is greater than the level of label expressed from the double stranded probe molecules; and

wherein the nucleotide probe molecules are further characterized by an ability to hybridize to target nucleotide sequences.

20. A method for monitoring the hybridization of target and probe by complementation, said method comprising of:

- a. incorporating fluorescent molecules into probes;
- b. detecting a first level of label in probe of step a;
- d. hybridizing a target with said labeled probe;
- e. detecting a second level of label after hybridization of probe and target;
- f. comparing the first and second levels of label between that of step b and that of step e, and wherein said difference between second and first levels is less than said first level of step b;
- g. washing of unhybridized target;
- h. repeating steps d - g until the difference between the first and second levels of label approaches approximately zero and/or about background levels.

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21. (Cancelled) A microarray substrate wherein the substrate is a bead, said bead having a surface area comprising attached probe molecules with a fluorescence label, said bead sizes range from about 10 microns to about 20 microns.

22. (Amended) A method for monitoring the hybridization of a probe and a target comprising, [providing] a fluorescently labeled probe [with a fluorescence label and] providing a detectable first level of fluorescence [of the labeled probe], and providing a detectable second level of fluorescence [of the labeled probe] when the labeled probe is hybridized to a complementary unlabeled target, wherein the second level is lower than the first level.

23. (Amended) A method for monitoring the hybridization of a probe and a target comprising supplying [providing] a fluorescently labeled probe [with a fluorescence label and] providing a detectable first level of fluorescence [of the labeled probe], and providing a detectable second level of fluorescence [of the labeled probe] when the labeled probe is hybridized to a complementary unlabeled target, wherein the second level is significantly lower than the first level.

24. (Amended) A method for monitoring the hybridization of a probe and a target comprising supplying [providing] a fluorescently labeled probe [with a fluorescence label and] providing a detectable first level of fluorescence [of the labeled probe], and providing a detectable second level of fluorescence [of the labeled probe] when the labeled probe is hybridized to a complementary unlabeled target, wherein the second level is approximately zero.

25. (Amended) A method for monitoring the hybridization of a probe and a target comprising supplying , [providing] a fluorescently labeled probe [with a fluorescence label and] providing a detectable first level of fluorescence [of the labeled probe], and a detectable second level of fluorescence [of the labeled probe] when the labeled probe is hybridized to a complementary unlabeled target, wherein the second level is approximately zero and the first level is greater than zero.

26. A substrate having a plurality of probes, wherein said probes are fluorescently labeled, the labeled probe providing a detectable first level of fluorescence.

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27. (Amended) A substrate having a plurality of probes, wherein said probes are fluorescently labeled, the labeled probe providing a detectable first level of fluorescence, and when hybridized to a complementary target providing a second level of fluorescence, wherein the second level is lower than the first level.

28. (Amended) A substrate having a plurality of probes, wherein said probes are fluorescently labeled, the labeled probe providing a detectable first level of fluorescence, and when hybridized to a complementary target providing a second level of fluorescence, wherein the second level is significantly lower than the first level.

29. (Amended) A substrate having a plurality of probes, wherein said probes are fluorescently labeled, the labeled probe providing a detectable first level of fluorescence, and when hybridized to a complementary target providing a second level of fluorescence, wherein the second level approaches zero.

30. (Amended) A substrate having a plurality of probes, wherein said probes are fluorescently labeled, the labeled probe providing a detectable first level of fluorescence, and when hybridized to a complementary target [level] providing a second level of fluorescence, wherein [the second level approaches zero and] the second level is greater than zero.

31. A substrate having a surface area, the surface area comprising attached labeled probe molecules, said probe further comprising a fluorescent label.

32. The method of claim 16, 17, 18, 20, 22, 23, 24 or 25, wherein the multiple labeled probes and the multiplying of the labeled probes are achieved by a non-amplification step.

33. (Added) A method of labeling or modifying a probe by incorporating an analog nucleotide.

34. (Added) The method of claim 33 whereby the labeled probe molecules are nucleotide analogs including 2-amino purine for adenosine or guanine; ribonucleoside or 2,6-diamino ribonucleoside, formycin A, formycin B, oxyformycin B, toyocamycin, sangivamycin, pseudouridine, showdomycin, minimycin, pyrazomycin, 5-amino-formycin A, 5-amino-formycin B or 5-oxo-formycin A for adenosine; 4-amino-pyrazolo [3,4d] pyrimidine,

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4,6-diamino-pyrazolo [3,4d] pyrimidine, 4-amino-6-oxo-pyrazolo [3,4d] pyrimidine, 4-oxo-pyrazolo [3,4d] pyrimidine, 4-oxo-6-amino-pyrazolo [3,4d] pyrimidine, 4,6-dioxo-pyrazolo [3,4d] pyrimidine, pyrazolo [3,4d] pyrimidine, 6-amino-pyrazolo [3,4d] pyrimidine or 6-oxo-pyrazolo [3, 4d] pyrimidine for cytosine or thymidine.

35. (Added) The method of claim 33 whereby the incorporated nucleotide analog is 2-aminopurine replacing adenosine or guanine nucleotides.

36. (Added) The method of claim 33 whereby after incorporation of the nucleotide analog including 2-aminopurine, the labeled probe is affixed on a solid substrate.

37. (Added) The method for quantifying the amount of a target molecule in solution comprising the steps of:

- a. incorporating a nucleotide analog including 2-aminopurine into a probe;
- b. affixing the labeled or modified probe on a substrate;
- c. detecting a first level of label expressed by the labeled or modified probe molecules on the substrate;
- d. contacting substrate with a volume of sample containing unlabeled or unmodified target molecules in solution;
- e. providing sufficient conditions and time for unlabeled or unmodified target molecules in solution to selectively pair and hybridize with the labeled probe molecules affixed on the substrate;
- f. removing the substrate and detecting the second level of label expressed by the labeled probed affixed on the substrate after exposure to the unlabeled or unmodified target molecules in solution;
- g. comparing the first and second levels of label expressed by the labeled or modified probe;

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h. identifying probe and target hybridized molecules by repeating steps c-f until the amounts of label expression between the first and second levels of label approaches zero and/or about background levels.